RMS *Titanic* Expedition – 2022 Draft Science Plan

Prepared for Participation in OceanGate Expeditions to the RMS *Titanic* wreck site and other North Atlantic Sites

RMS *Titanic* Expedition – 2022 Science Plan
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The discovery of the wreck of RMS *Titanic* at about 3,800 m south-southeast of Newfoundland in 1985 led to a series of expeditions over the next 20 plus years. The wreck and its debris field were described and mapped (Figure 1), local geology and oceanography described, and parts of the wreck were salvaged. Interestingly, the wreck site is in the general vicinity of a massive undersea earthquake and subsequent slump failure in 1925, 13 years after *Titanic* sank. However, aside from a few publications (e.g., new species of bacteria, description of growth of a single coral, fauna near the wreck), the biology and ecology of the wreck and surroundings, as well as earthquake impacts, are poorly known. It was proposed that the large delivery of iron (a limiting nutrient in the deep sea) to the seabed might promote increased productivity. Thus, the wreck site may support an "island effect," which may have a limited duration as the wreck deteriorates rapidly. Just the wreck structure alone added considerable habitat complexity to the area. This site seems quite valuable for biological investigations on deep-sea benthic communities. The site is also useful for comparisons with other regional sites that have been well studied (e.g., Mid-Atlantic Ridge, The Gully canyon). In addition to new data collected in the Ocean Gate Expeditions, we will utilize past imagery and other data for temporal comparisons of communities and to assess faunal changes. We have developed a working draft of faunal lists and photos of animals we expect to see or have seen, and this will be available to the cruise participants (See example Appendix A). The list and images will be updated and improved as new data are added.

The 2022 expeditions will use St. John's, Newfoundland as the port of call, and all missions will use the MV *Horizon Arctic* (see https://oceangateexpeditions.com/blog/horizon-arctic). There will be five 8-day missions (Legs) with a sixth mission optional. One day in port between missions will be used to exchange crews. In general, we anticipate one submersible dive per day on or near the *Titanic* site, using the 5-person submersible *Titan*. Submersible crew will likely consist of one Pilot in charge of all submersible operations, one Lead Scientist and three Mission Specialists. Although all dive data will be electronically archived, we will complete a paper copy dive log sheet for each dive (Appendix B). The dive Lead Scientist and Chief Scientist, assisted by the Mission Specialists, will complete these Station Logs during and after each dive. Each dive will emphasize collection of science quality high-definition video as well as collecting water samples using Niskin bottles for DNA analysis. General guidelines for submersible science operations and eDNA are provided in Appendices C and D, respectively.

For science personnel not participating in a dive (on deck or undersea) and for times when the *Titan* is not diving (night, bad weather, repairs), we anticipate there will be data and sample activities. These may include labeling and treatment of water samples, video and photo archiving, preliminary analysis of video, and completing Station Logs.

This science components of this cruise are a collaborative effort between OceanGate Expeditons, OceanGate, Inc., OceanGate Foundation, eDNAtec, Nortek, and academic partners Univ. of NC at Wilmington, Edinburgh Univ., and Univ. of Rhode Island.

Mission Legs and Science Personnel

Leg I - 15-23 June: Beverly McClenaghan (eDNAtec, Centre for Environmental Genomics Applications), Science Lead for genetic sampling and analysis and Science Lead for Leg I; Chris McCabe (Univ. of Rhode Island), GIS activities. Assisted by Alyx Terrell (Ocean Gate) and Mission Specialists.

Leg II – 23 June - 1 July: Dr. Anna Gebruk (Changing Oceans Research Group, School of GeoSciences, Univ. of Edinburgh), Science Lead for Leg II; Beverly McClenaghan, Science Lead for

DNA work; Dr. Bridget Buxton (Univ. of Rhode Island), Lead Archaeologist; assisted by Alyx Terrell and Mission Specialists.

Leg III -1-9 July: Dr. Anna Gebruk, Science Lead for Leg III; Dr. Bridget Buxton, Lead Archaeologist; assisted by Alyx Terrell and Mission Specialists.

Leg IV – 9-17 July: Dr. Steve W. Ross (Univ. of NC at Wilmington), Expedition Chief Scientist; Dr. Rod Mather (Univ. of Rhode Island), Lead Archaeologist; assisted by Alyx Terrell and Mission Specialists.

Leg V – 17-25 July: Dr. Steve W. Ross, Expedition Chief Scientist; Dr. J. Murray Roberts (Head Changing Oceans Research Group, School of GeoSciences, Univ. of Edinburgh), biodiversity and deep-sea expert. Assisted by Alyx Terrell and Mission Specialists.

Leg VI (optional) – 25 July – 2 August: Alyx Terrell and Mission Specialists.

Onshore science support will be provided by eDNAtec, Nortek, Univ. of Rhode Island, and Univ. of Edinburgh (including Dr. Lea-Anne Henry). Other scientists with varying expertise will be included as need arises.

Science Objectives [bullets can be expanded as needed.]

1. Characterize overall fauna on the wreck and in the near vicinity, documenting species composition, habitat utilization (e.g., preferential use of the wreck vs open bottom), trophic position, and size structure. Sampling will rely most heavily on HD video and still photography. Benthic data will be emphasized although observations will also include the water column. Fishes (SWR lead) and deep-sea corals (JMR, L-AH leads) will be areas of concentration, considering their importance and general lack of information. Deep-sea biofouling communities will be an additional focus (AG lead).
2. Conduct broad spectrum biodiversity surveys via environmental DNA analysis (BMcC lead), which supports Objective #1. Collect water samples for analysis of eDNA (see Appendix D). Most such samples will come from the bottom, but water column samples may be needed. These would likely be collected by 5-L Niskin bottles triggered by the Titan submersible (2 samples per dive). Triplicate 1.5 L water samples will be collected from each Niskin bottle and filtered through 0.2 µm Sterivex™ filters using a suitable pump (likely a peristaltic pump). Processing and filtration must be in an area kept clean and free of any biological material that could contaminate the samples. The filters must be immediately frozen (-20°C) and remain frozen to preserve the DNA. Sediment samples will be opportunistically collected from the submersible skids and frozen for DNA analysis (Appendix D).
3. Collect physical oceanographic data to characterize wreck environment, local hydrography, and water masses of the region. Such data needed include water column and benthic measurements of temperature, salinity, dissolved oxygen, currents. Water column and benthic profiles will be collected on each dive by a CTD attached to the submersible. The MIDAS SVX2 Combined CTD/SVP will be used to collect sound velocity, conductivity, temperature, and pressure of the environment around the wreck and through the water column (AT lead). This instrument will remain attached to the submersible throughout the expedition and can be paired to the Nortek Aquadopp for concurrent measurements.
4. Nortek will partner with us and provide two different models of the Nortek Aquadopp current meter, which at minimum will measure bottom water current speed and direction and temperature. Test deployments may occur on Leg I, with deployment of the meters near the wreck site on Leg

II. Meters will be retrieved on Leg V. Comparisons between data from the two meters will be conducted post-cruise.

5. Continue with coral dispersal modelling (L-AH lead) and will greatly benefit from #3 above. This objective will mostly be conducted on shore.
6. Characterize and map benthic habitats at and near the wreck site, adding to and improving existing GIS maps. This will require a track map of the submersible's dive tracks. Biology (#1 above) can be overlaid onto the habitat maps. Compare lists of benthic fauna (both biofouling and natural substrates) with earlier observations known from literature and expedition reports. Assess for community change.
7. Continue archaeological investigation of the wreck site, noting and mapping wreck parts and debris and documenting wreck deterioration. If possible, any sediments attached to the submersible can be measured for pH. The same sediment samples collected for eDNA (#2 above) can be used for this. Precision measurement of pH (later on shore) will provide additional habitat information as well as data related to the wreck deterioration. One caveat is that mixing of sediments or dilution by surrounding water may impact the accuracy of these measurements.
8. We will attempt to collect water samples for carbonate chemistry analysis. These can be taken by Niskin bottles. Brief methods are provided in Appendix E.

RMS *Titanic* Expedition – 2022: Gear/Equipment and Capability Needs (wish list)

High quality video (with zoom, pan and tilt capability using sub)
Niskin bottles (SWR suppled 2 5-L bottles) and ability to trigger Niskin bottles from the sub
eDNA processing: standard freezer (-20°C)
Full CTD instrumentation on the sub (Temp, Depth, Sal, Sound Velocity, DO, +?)
ADCP mounted on ship and/or sub for data on local currents
Nortek Aquadopp current meters for bottom deployments

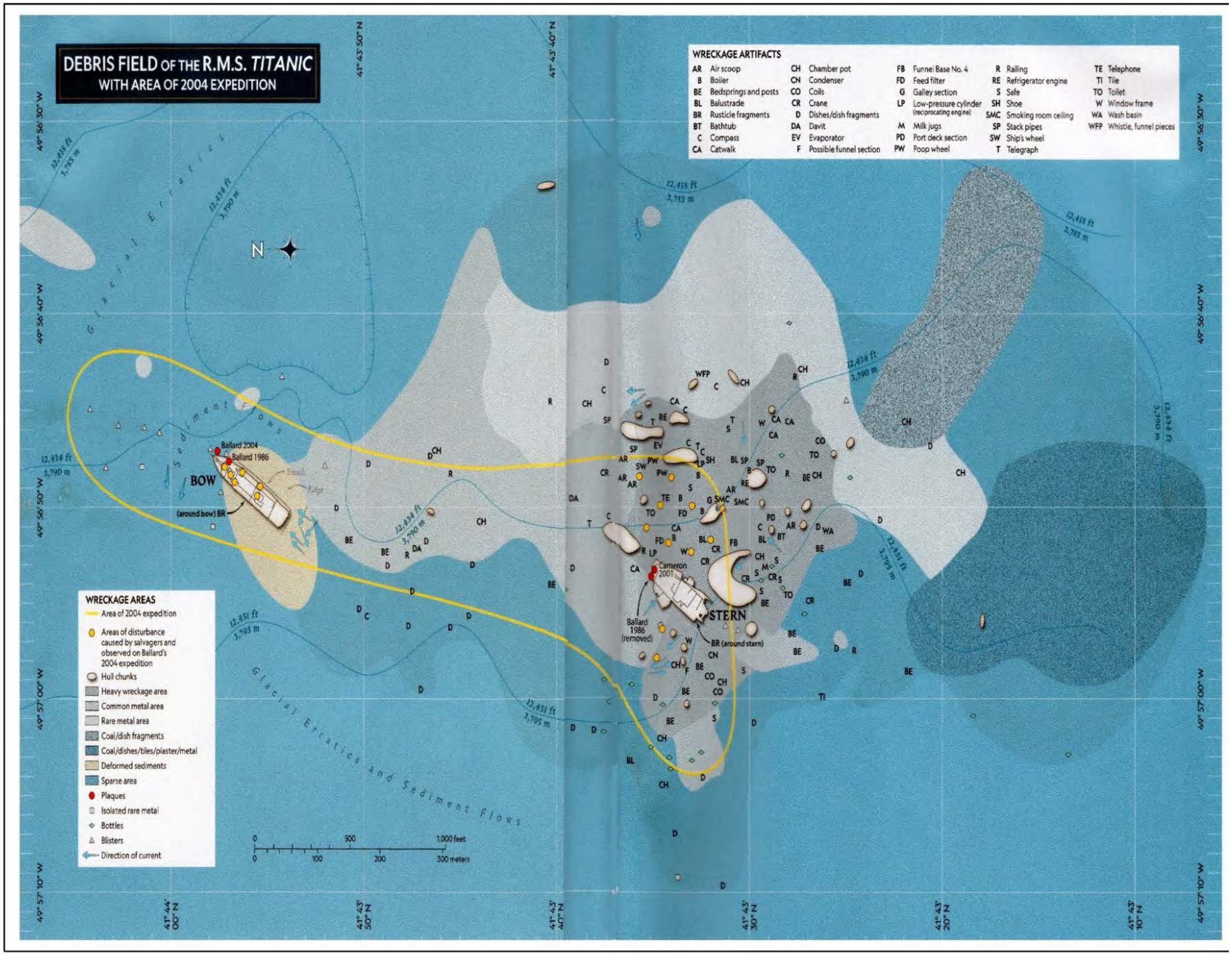
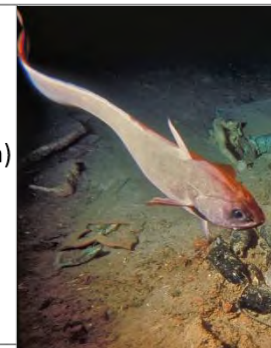


Figure 1. RMS *Titanic* wreck site (original source via National Geographic Society, ca. 2004).

APPENDIX A

Biodiversity identification matrix – examples of common taxa

(A. Gebruk, S.W. Ross, J.M. Roberts, L-A Henry, May 2022)

	**Cnidaria** Octocorallia *Chrysogorgia agassizii* © WoRMS		**Porifera** Hexactinellida *Caulophacus* sp. (sponge) © NOAA		**Echinodermata** Brisingidae Gen. sp. (Brisingid starfish) © Galkin et al., 2002		**Fish** Gadiformes *Coryphaenoides cf rudis* (rattail) © OceanGate
	**Cnidaria** Octocorallia *Lepidisis* sp. (bamboo coral) © OceanGate		**Porifera** Demospongiae Gen. sp. (sponge) © wikipedia.org		**Echinodermata** Ophiurida Gen. sp. (brittle stars) © OceanGate		**Fish** Aulopiformes *Bathysaurus* sp. (lizardfish) © OceanGate
	**Cnidaria** Hexacorallia Actiniaria Gen. sp. (sea anemone) © OceanGate		**Arthropoda** Decapoda Benthesicymidae (shrimp) © OceanGate		**Arthropoda** Decapoda *Munidopsis* sp. (squat lobster) © OceanGate		**Fish** Anguilliformes *Nemichthys* sp. (snipe eel) © WoRMS

APPENDIX B

HOV Titan 2022: Submersible Station Log Page 1 of ____

Station #	Date (dd/mmm/yy)	Science Observers	Dive Times (DST)		Total Bottom Time (min):
Titan-2022-		1. 2. 3.	Time at Launch:	Time at Surface:	
Bottom Depths (m)			Time Arrive Bottom:	Time Leave Bottom:	
Depth at Launch:	Depth at Surface:				
Depth on Bottom:	Depth Leave Bottom:		CTD File Name:		

Wind Speed:	Wind Direction:	Air Temp:	Seas:	Sky:	DGPS ☐

Launch Latitude:	Launch Longitude:	At Surface Latitude:	At Surface Longitude:
° N	° W	° N	° W
On Bottom Latitude:	**On Bottom Longitude:**	**Leave Bottom Latitude:**	**Leave Bottom Longitude:**
° N	° W	° N	° W

Gear Used:	Cameras Used:

Sequential Events Log

Video Time	Depth (m)	Description

Events Log (Con't) **Station #:Titan-2022-______ Observer:______ Position:______ Page ___ of ___**

Video Time	Depth (m)	Descripton

APPENDIX C

ISSUES TO CONSIDER FOR UNDERSEA VEHICL SCIENCE OPERATIONS
(S.W. Ross, revised April 2022)

Included are observations (and requirements) for how we conduct submersible or ROV dives, mostly related to audio and video data. This is most relevant to the scientist in charge of a dive, but all participants share responsibility. Operations are subject to modification as needed. Please review these.

The lead for science operations for a dive must be prepared to make decisions and understand the priorities of the dive as well as the mission overall. The other Mission Specialist are charged with tracking all activities and reminding the lead scientist of dive requirements as necessary. When in doubt, communicate with the chief scientist and pilot.

Mission Specialists should take copious notes (using audio recorders, computers and/or hard copy forms) on all observations, regardless of whether they seem important. Divers are responsible for learning how to operate science gear in their charge. The lead scientist should be in charge and operate the science video and/or still cameras. Practice with the gear (cameras & recorders) to the extent possible before your dive.

We usually have multiple objectives to accomplish during the expedition. Therefore, priorities for each dive will be established before the dive and given to the science dive team. A few things like transects, digital still images, general videography, and collecting water samples will be objectives for every dive. Other things, like deploying equipment may be done more selectively. We need to make sure that the submersible logs the positions for any major event, every gear deployment, and any noteworthy bottom and wreck features. In the past the verbal notes of some observers have been marginal in many ways and difficult to hear on recordings. Speak clearly and with some volume; your voice is potentially being recorded to several devices (cameras and audio recorders). The audio recorder should be run through most, if not all, of the dive to obtain a complete record. Start any recording with date, time, dive number and your name. The main camera is usually turned on close to the bottom or in the water column depending on what is observed. More details on video are below.

Video and audio operations
Our video archive often is not of the quality that it could be. A few simple things can improve these data significantly. Since the submersible pilot runs most of the gear, one of the main responsibilities of the lead scientist is the video operations. Suggestions below are based on the problems found in past video data.

If samples are collected, it is important to film what you are collecting, with all cameras when possible. Often in the past the camera is looking away and the verbal commentary is inadequate. Filming the sample area prior to and during collecting is critical for tracking samples, and a powerful way for us to increase our information about the samples.

There are often too many close-ups (tight zooms) that are of poor quality (usually out of focus or moving too much) or are repetitious. While we do need good close-ups, we should discuss as we go what is being filmed. Monitor both focus and camera movement. Anticipate the vehicle's movement and its effect on the camera view. In addition to the close-up problem, there is sometimes a general lack of quality control on focus and camera angle. Monitoring the cameras and knowing how to run them are vital. If you cannot focus the camera or detect lens fogging, discuss this with the pilot.

We have often ended up with a lot of "dead footage" - minutes of looking at the same thing while the vehicle is doing something else. One problem with this is that the verbal commentary is so sparse or of

poor quality, that we cannot later determine what was happening. Always be aware of what the cameras are doing (check them regularly) and adjust as needed. Use the zoom slowly and smoothly. Also, move the camera smoothly and slowly. If the vehicle is moving too much or too fast, ask the pilot to adjust. At any time you can ask the pilot to help position the vehicle more effectively. Since the Titan main video camera is a fixed mount on the lower right front of the vehicle, it is critical to work with the pilot to position images where needed.

If the video screen or other readouts display data overlays, we need to monitor and read out those values to the audio recorders frequently. As a backup and to make the audio records most useful, it is very important to record on the audio regular readouts of time, depth and temperature as seen on the video (or other) screen. **The most important things to record frequently are time and depth.** In general, there has not been enough audio commentary on the dive recordings. A viewer later has a very difficult time trying to determine what was going on. Why is the vehicle not moving? What activities are being done? Audio sometimes was barely picked up on the main video recorder. Speak clearly. Also, there has not been enough audio commentary on the wider area surrounding where the vehicle is working. Describe what you see in detail.

Try to understand what you are seeing in the context of data needs. Some species need more detailed video documentation, some less. Keep in mind deep-sea animals are usually very difficult to identify with only visual images. Thus, high quality video from different angles and zoom settings are needed to assist identifications.

Often when the vehicle is moving large distances, as when moving toward a reef, it is too far off the bottom or moving too fast, and the resulting camera video quality is poor. Try to make all video count as much as possible. If video cannot be improved because of the way the vehicle needs to move, this is a time when accurate, detailed audio recordings are most needed.

When on transect runs, make sure the main camera lens is set on wide angle. Tell the pilot where to go before starting & how the run is supposed to go (either straight by a compass course-often hard on the reefs, or following a depth or the bottom contour). Keep the speed slow (< 1 knot) and distance off bottom as small as practical. Make abundant audio notes, especially of time, depth, & temperature. Note estimated lateral visibility. Request lat/long locations at beginning and end of transects. We will discuss transects aboard ship in more detail. Since *Titanic* missions have many objectives, the pilot will influence transect operations.

APPENDIX D

Environmental DNA Biodiversity Sampling (Beverly McClenaghan, May 2022)

Environmental DNA (eDNA) can be collected from the environment to identify species present in an ecosystem. Multicellular organisms constantly shed cells containing DNA into their environment (skin cells, feathers, hair, feces, urine, saliva, etc.) which can be collected for analysis. Environmental genomics methods are extremely sensitive, and like forensic DNA approaches, unique DNA signatures are used to identify all the organisms. A small amount of sediment or water can contain DNA from hundreds or thousands of organisms living in that ecosystem. But because of its high sensitivity, it is also extremely sensitive to contamination. A single bit of soil or water from an unintended source can contaminate an eDNA sample. Sterile technique must be used when handling eDNA samples and equipment for eDNA sampling. Follow eDNAtec's standard operating procedures when participating in the collection, filtration, and storage of eDNA samples.

The overall goal of the eDNA sampling is to characterize the biodiversity on the wreck and compare the wreck biodiversity to that of nearby habitats to better understand the ecological community associated with the wreck. To accomplish this goal, eDNA samples will be collected from multiple habitat types outlined below.

- On Wreck (wreck parts always in view)
    - Main Structure
    - Debris Field
- Off Wreck (Determining this may become somewhat arbitrary, but the goal is to not have any wreck structure within sight of sample. Existing wreck area maps, as below, may guide this but is subject to influence by submersible dive plan)
    - Up current control site
    - Water column

eDNA sampling will consist primarily of water sample collection. Water samples will be collected using Niskin bottles fixed to the exterior of the Titan submersible, which can be triggered from the sub. Niskin bottles will be closed at the desired sampling locations. All samples should be taken as close to sediment/structure as possible. The time, location, depth, water temperature and any habitat features associated with the sample collection should be noted. The proposed baseline eDNA sampling plan is outlined below.

- Baseline eDNA Sampling Plan (total: 48 eDNA water samples plus negative controls):
    - 4 bow sites
    - 4 stern sites
    - 4 debris field sites
    - 4 off wreck sites (increase number if possible)

At a minimum, samples from these sites should be collected. Depending on dive plans and objectives, additional sites should be sampled whenever possible. Both Niskin bottles should be deployed and used on each dive to maximize the number of sites sampled. Potential additional sampling sites are outlined below.

- Increase # sites in each habitat type (prioritize additional off-wreck sites, up to 12 sites)
- Collect in water column above the wreck, and off wreck

    - up to 16 sites
- Distance transect away from wreck

Some dives may target sampling locations other than the *Titanic* wreck. eDNA samples should also be collected on dives to these other locations as well. Depending on the dive location and objectives, eDNA samples may be collected in the water column, close to the bottom, or close to another habitat feature. The time, location, depth, water temperature and any habitat features associated with the sample collection should be noted. Other potential sampling locations may include:

- Transatlantic cables
- Other shipwrecks (e.g., “mystery” shipwreck from similar time period)
- Continental shelf
- Canyons

When the sub surfaces from a dive, the eDNA water samples must be collected from the Niskin bottles. Three 1.5 L subsamples will be collected from each Niskin bottle. Each of these samples will be filtered in a clean area *as soon as possible* after collection. Filters will be stored frozen at -20°C in the ship’s freezer to preserve the DNA.

eDNA sampling will also include opportunistic sediment sampling. Sediment will be collected from the skids of the submersible, which accumulate sediment when landing on the ocean floor. Each time sediment samples are collected from the skids, triplicate ~5g samples will be collected and frozen. Sediment sampling locations will depend on dive objectives and where the sub lands, but it would be desirable to get sediment samples from:

- very close to wreck
- debris field
- off wreck

APPENDIX E

Water sampling for Total Alkalinity (TA) and Dissolved Inorganic Carbopn (DIC) analysis (J. Murray Roberts, May 2022)

Equipment needed:
EPA vials 40ml
Pipette and Pipette tips
Salinity/ temperature meter (should be able to get salinity and temperature from CTD)
Mercuric chloride, HgCl (saturated solution)
Disposable gloves
Waste container for used tips
Lab coat
Safety glasses
Pens
Kimwipes
Silicone tubing
Benchcote (To put on bench in area where you will add mercuric chloride. Dispose of at end of cruise)

1. Wait for Niskin bottles to return to deck.
2. Attach silicone tubing to drain port of Niskin bottle to be sampled.
3. Open water flow and rinse sample bottle twice.
4. Fill sample bottle with water, leaving no air space.
5. Screw lid on bottle and label accordingly.
6. Transport sample bottles to lab/ area where HgCl will be added.
7. Remove the lids and immediately poison with a saturated solution of HGCL (50 µl for 40 ml vials) in order to prevent any further biological activity in the stored sample.
8. Replace lids.
9. The sample should be shaken thoroughly to mix the HgCl homogeneously.
10. Samples should be stored in a cool and dark place.
11. The water salinity and temperature at the time of water collection are also required. Dissolved oxygen measurement is desirable.